Musselbrook Reserve Scientific Study 1995

Survey for protozoan parasites in native animals from Musselbrook Reserve, Lawn Hill National Park, Queensland

Peter O'Donoghue and Eric Vanderduys
Department of Parasitology, The University of Queensland, Brisbane Q 4072

Abstract: An opportunistic survey for protozoan parasites was conducted on native animals caught in Musselbrook Reserve during The Royal Geographical Society of Queensland expedition in June 1995. Samples were collected from 98 animals belonging to 43 different species. Protozoan parasites were detected in 13 individual animals (13.3%) belonging to 7 separate species. The results confirmed previous reports of the ciliate *Nyctotherus* in green tree frogs (*Litoria caerulea*) while the remainder constituted new host records of infection, including: *Nyctotherus* in the desert tree frog (*Litoria rubella*); the eimeriorine coccidian *Eimeria* in the dtella (*Gehyra dubia*); the eimeriorine coccidian *Schellackia* and the adeleorine coccidian *Haemogregarina* in olive pythons (*Liasis olivaceus*); *Haemogregarina* in Burton's legless lizard (*Lialis burtonis*); *Haemogregarina* in the robust dtella (*Gehyra robusta*); and the haemosporidian *Haemoproteus* in the ring-tailed dragon (*Amphibolurus caudicinctus*).

INTRODUCTION

Information on the taxa of protozoan parasites occurring in native Australian animals is sparse and fragmentary. Most records are confined to miscellaneous case reports and incidental findings in the course of other studies. Several small surveys were conducted on individual host groups, mainly reptiles, amphibians and birds, by our pioneering parasitologists (Johnston & Cleland 1910; Cleland & Johnston 1910; Johnston 1912) and their results have been catalogued and reviewed (Mackerras 1958, 1961). Veterinary parasitologists have subsequently conducted extensive studies on pathogenic protozoan species mainly in introduced domestic and companion animals (Callow 1984) and in large native animals, especially macropodid marsupials (Barker et al. 1989). In addition, most studies have been conducted on animals from eastern and southern Australia and few studies have included animals from tropical northern Australia. Comprehensive studies have yet to be conducted on the protozoan parasites of most species of native Australian animals, especially those occupying relatively undisturbed natural habitats. The invitation to participate in the Musselbrook Reserve Scientific Study, organised by The Royal Geographical Society of Queensland in association with the National Parks Association of Queensland, presented an ideal opportunity to examine a wide range of native animals from this remote geographic location.

MATERIALS AND METHODS

All sampling was conducted in the Musselbrook Reserve, Lawn Hill National Park, Queensland (18°36'S, 138°08'E) under permit by the Queensland Department of Environment and Heritage (no. H1/000044/95/SAA) and The University of Queensland Animal Ethics Committee (no. PARA/252/95/D). All animals were caught on-site by field staff during June 1995. Reptiles and amphibians were caught by hand or pit-traps, birds and bats by mist-netting, and cage-traps were set for small terrestrial mammals. Where possible, all animals were released at the site of capture after samples had been collected. Faecal samples were collected by abdominal massage or by housing the animals in a suitable container and allowing them to void naturally. Faecal samples were stored in equal volumes of 2% potassium dichromate and transported back to the laboratory. Small-volume blood samples were collected from larger animals by peripheral venepuncture and

from smaller animals by tail or toe clip. Thin blood smears were prepared on-site, air-dried and transported back to the laboratory. Faecal samples were examined for protozoan cysts and oocysts following centrifugal flotation in saturated magnesium sulphate solutions. Blood smears were fixed in methanol immediately prior to staining with Giemsa (pH 6.8). All samples were examined by light microscopy at 100-1,000x magnification and any protozoan organisms encountered were photographed and then measured using a calibrated eye-piece graticule. Morphometric and statistical analyses were conducted using the Statistix® computer program.

RESULTS

Prevalence of infections

Samples were collected from a total of 98 animals (2 bats, 37 lizards, 9 snakes, 4 tortoises, 23 frogs and 23 birds) belonging to 43 different species (complete list given in Appendix 1). Protozoan parasites were detected in 13 individual animals (prevalence of 13.3%). Infections were detected in 2 species of frogs, 4 species of lizards and one snake species. Five different genera of parasitic protozoa were detected (Table 1).

Table 1. Protozoan parasites detected in animals from Musselbrook Reserve

Parasite	Stage detected	Location	Host species	no. infected/ no examined
Polyhymenophorea				
Nyctotherus	cysts	faeces	*Litoria caerulea* (green tree frog)	3/6
Nyctotherus	cysts	faeces	*Litoria rubella* (desert tree frog)	1/1
Coccidea				
Eimeria	oocysts	faeces	*Gehyra dubia* (dtella)	1/2
Schellackia	sporozoites	blood	*Liasis olivaceus* (olive python)	2/3
Haemogregarina	gamonts	blood	*Liasis olivaceus* (olive python)	2/3
Haemogregarina	gamonts	blood	*Lialis burtonis* (Burton's legless lizard)	1/1
Haemogregarina	gamonts	blood	*Gehyra robusta* (robust dtella)	2/7
Haematozoea				
Haemoproteus	trophozoites & gamonts	blood	*Amphibolurus caudicinctus* (ring-tailed dragon)	1/3

Enteric protozoa

Developmental stages of 2 types of protozoa were detected in faecal samples; ciliate cysts from frogs and coccidian oocysts from a gecko (Table 1). The protozoan genera involved were identified on the basis of their unique morphological characteristics.

Cysts of the heterotrichous ciliate *Nyctotherus* were detected in 3 green tree frogs and one desert tree frog (Table 1). Cysts from green tree frogs averaged 85.8 μm in length by 73.6 μm in width and those from the desert tree frog averaged 84.0 μm by 71.2 μm (Table 2). No significant differences were observed in the mean size, shape or appearance of the cysts recovered from the different frogs. The cysts were ovoid in shape, dark brown in colour and were surrounded by a thick cyst wall with a distinct thin polar plug. The cysts contained single light-brown trophozoites with prominent transverse striations corresponding to the rows of somatic kineties (Fig. 1).

Table 2. Morphometric characterization of enteric protozoa. (x = mean, SE = standard error, CV = coefficient of variation, min = minimum, max = maximum, n = number of observations, measurements in μm)

Parasite	Host	Character	x	SE	CV	min	max	n
Nyctotherus	*L. caerulea*	cyst length	85.8	1.64	10.5	72	108	10

		cyst width	73.6	1.60	12.2	60	96	10
Nyctotherus	*L. rubella*	cyst length	84.0	1.16	8.7	76	96	10
		cyst width	71.2	1.72	7.5	64	80	10
Eimeria	*G. dubia*	oocyst length	32.1	1.02	10.0	28	38	10
		oocyst width	19.7	0.60	9.6	17	23	10
		sporocyst length	13.3	0.47	11.2	11	16	10
		sporocyst width	7.3	0.30	13.0	6	9	10

Oocysts of the eimeriorine coccidian *Eimeria* were detected in a single dtella (Table 1). Unsporulated oocysts contained a single granular sporoblast whereas sporulated oocysts contained 4 sporocysts (Fig. 2). The oocysts were elliptical in shape with average dimensions of 32.1 µm by 19.7 µm (Table 2). They were surrounded by a thin smooth oocyst wall without a micropyle and they did not contain polar granules or residual bodies. The sporocysts were ovoid with average dimensions of 13.3 µm by 7.3 µm (Table 2). Each sporocyst contained 2 elongate curved sporozoites and a granular residual body but Stieda bodies were not observed. The sporozoites contained large refractile globules in their posterior ends.

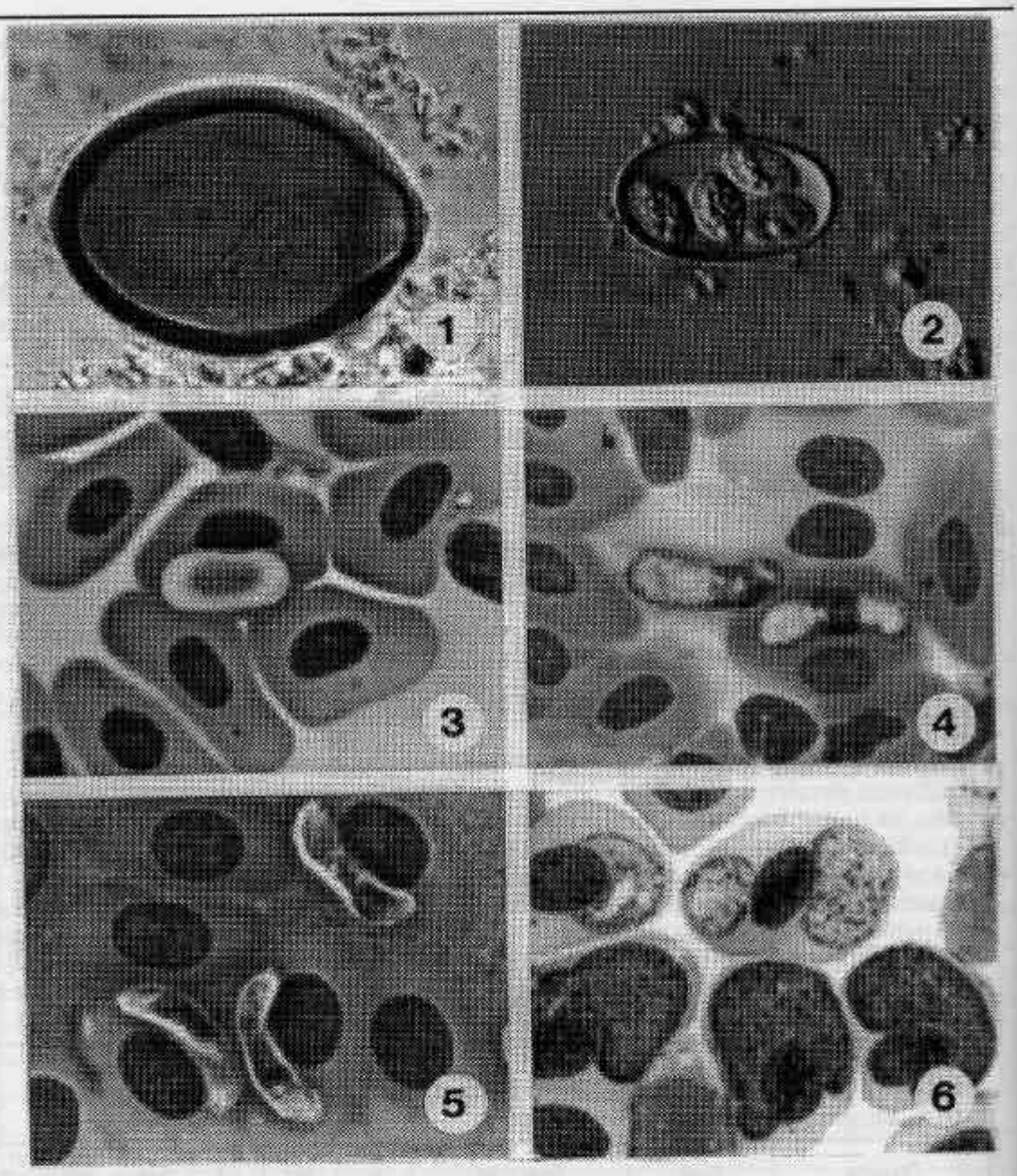
Haemoprotozoa

Different developmental stages of 3 types of haemoprotozoa were detected in the blood smears, including coccidian sporozoites in snakes, coccidian gamonts in snakes and lizards and haemosporidian trophozoites and gamonts in a dragon (Table 1). The parasites were identified to genus by their distinctive morphological features.

Sporozoites of the eimeriorine coccidian *Schellackia* were detected in erythrocytes from 2 olive pythons (Table 1). The sporozoites were oblong in shape and appeared as pale vacuolated bodies (Fig. 3) with average dimensions of 11.0 µm in length by 5.2 µm in width (Table 3). They were located in the erythrocyte cytoplasm adjacent to the host cell nucleus and infected cells exhibited some lateral distention.

Table 3. Morphometric characterization of haemoprotozoa. ($\bar{x}$ = mean, SE = standard error, CV = coefficient of variation, min = minimum, max = maximum, n = number of observation, all measurements in µm)

Parasite	Host	Character	$\bar{x}$	SE	CV	min	max	n
Schellackia	*L. olivaceus*	sporozoite length	11.0	0.28	11.3	9.7	15.5	20
		sporozoite width	5.2	0.08	6.7	4.8	6.0	20
Haemogregarina	*L. olivaceus*	gamont length	16.2	0.74	20.4	12.0	20.4	20
		gamont width	5.0	0.17	15.5	3.6	6.0	20
Haemogregarina	*L. burtonis*	gamont length	13.3	0.48	11.3	11.0	14.9	10
		gamont width	3.6	0.11	9.5	3.1	4.0	10
Haemogregarina	*G. robusta*	gamont length	15.2	0.21	6.2	13.5	16.8	20
		gamont width	4.2	0.09	10.2	3.4	4.8	20
Haemoproteus	*A. caudicinctus*	trophozoite length	7.1	0.30	13.5	5.4	8.2	10
		trophozoite width	4.0	0.20	16.2	3.0	5.6	10
		gamont length	12.8	0.53	13.1	11.0	15.8	10
		gamont width	5.7	0.63	35.3	3.2	9.2	10



Figures 1-6. Light micrographs of parasitic protozoa detected in native animals from Musselbrook Reserve. Fig. 1. Ovoid cyst of *Nyctotherus* detected in faeces of desert tree frog (*Litoria rubella*). x430. Fig. 2. Elliptical oocyst of *Eimeria* harvested from faeces of dtella (*Gehyra dubia*). x720. Fig. 3. Vacuolated sporozoite of *Schellackia* in erythrocytes from olive python (*Liasis olivaceus*). x1,200. Fig. 4. Elongate gamont of *Haemogregarina* in erythrocytes from olive python (*Liasis olivaceus*). x1,000. Fig. 5. *Haemogregarina* gamonts in erythrocytes from Burton's legless lizard (*Lialis burtonis*). x1,200. Fig. 6. *Haemoproteus* trophozoites (pale) and gamonts (dark) in erythrocytes from ring-tailed dragon (*Amphibolurus caudicinctus*). x1,200.

Gamonts of the adeleorine coccidian *Haemogregarina* were also detected in erythrocytes from 2 olive pythons (Table 1).The gamonts appeared as elongate crescent-shaped bodies lying adjacent to the host cell nucleus (Fig. 4). They measured on average 16.2 µm in length by 5.0 µm in width (Table 3) and consisted of a pale basophilic vacuolated cytoplasm with a dense centrally-located nucleus. Infected cells were slightly enlarged and the gamonts displaced the host cell nucleus to one side. Haemogregarines were also detected in a robust dtella (Table 1). They were similar in appearance to those from the snakes except that they were slightly smaller in size with average dimensions of 15.2 µm by 4.2 µm (Table 3). Numerous haemogregarines were also detected in a Burton's legless lizard but they were different in size, shape and appearance to those from the snakes. They were less robust and smaller in size with mean dimensions of 13.3 µm by 3.6 µm (Table 3). The gamonts appeared to have shrunk within cytoplasmic vacuoles located immediately adjacent to the host cell nuclei (Fig. 5). They contained a basophilic cytoplasm and an elongate dense nucleus. Infected cells were slightly enlarged but the host cell nucleus was not displaced to one side.

Gamonts and trophozoites of the haemosporidian parasite *Haemoproteus* were detected in erythrocytes from a ring-tailed dragon (Table 1). The parasites appeared as ovoid to polymorphic bodies located in the erythrocyte cytoplasm next to or around the host cell nucleus (Fig. 6). All stages were speckled in appearance due to presence of dark refractile haemozoin pigment. Trophozoites were evident as small pale-staining ovoid to elliptical bodies with mean dimensions of 7.1 µm by 4.0 µm (Table 3). Gamonts appeared as larger dark-staining U-shaped bodies measuring on average 12.8 µm by 5.7 µm (Table 3). The gamonts were wrapped around the host cell nucleus and frequently displaced it to one pole. Occasionally, the gamonts could be differentiated into macrogamonts and microgamonts by their pale blue and pale red cytoplasms respectively.

DISCUSSION

Infections by protozoan parasites were detected in 13.3% of the 98 animals examined. They were detected in 4 of 23 frogs, 5 of 37 lizards and 4 of 9 snakes but not in any of 23 birds, 4 tortoises or 2 bats. Although the small number of animals involved prohibits any meaningful analyses on the prevalence of infections in individual host groups, it is interesting to note that all infections were detected in terrestrial reptiles and amphibians. This apparent bias in the occurrence of infections may simply be circumstantial or it could indicate that parasites occur more frequently in these particular host assemblages in this region. Not all infections may have been detected because diagnostic tests were only conducted on faecal and blood samples. Additional infections may have been detected if comprehensive post-mortem examinations had been conducted on all host organs and tissues. Nevertheless, infections by 5 different genera of protozoan parasites were detected in 7 host species. Most of these results constitute new host records of infection and all extend the geographic range of infections.

The ciliate *Nyctotherus* has previously been reported in green tree frogs collected around Sydney and Brisbane but no measurements or descriptions were included in the original reports (Cleland & Johnston 1910; Johnston 1916). The ciliate has not previously been detected in the desert tree frog. Although we detected only cysts and not trophozoites in faecal material, the parasites are not to be confused with opalinids commonly found in amphibian hosts, because the cysts are unique in their appearance. Nonetheless, detailed silver impregnation studies on trophozoites are required to identify the species of *Nyctotherus* infecting tree frogs in Australia.

Coccidia are common protozoan parasites in many vertebrate animals and it was surprising to detect *Eimeria* infections in only one animal. This result, however, does constitute a new host record of infection as coccidia have not previously been reported in the dtella (*Gehyra dubia*). The oocysts were similar in morphology to those of *E. gehyrae* described from *Gehyra variegata* and to those of *E. egerniae* described from the gall bladder of the skink *Egernia whitii* by Cannon (1967). They were also similar in shape to those of *E. gastrosauris* described from the stomach of *Heteronotia binoei*, *Oedura monilis* and *Gehyra australis* by Paperna (1994) but they lacked oocyst residual bodies and were slightly smaller in size. Further studies are required on these reptilian coccidia to establish reliable diagnostic characters, describe their endogenous developmental cycles and determine the extent of their host specificity.

Examination of thin blood smears revealed the presence of *Schellackia* developmental stages in erythrocytes from olive pythons. These parasites have previously been recorded in various amphibians and reptiles, particularly in lizards. They complete their entire developmental cycle (merogony, gamogony and

sporogony) in the small intestinal epithelium, connective tissues or reticulo-endothelial system culminating in the formation of intraerythrocytic sporozoites (cf. Paperna & Ostrovska 1989). These stages are ingested by blood-sucking invertebrates (mainly mites) but they do not undergo further development in the vectors. Infections are transmitted to new vertebrate hosts when they ingest infected mites. While the detection of sporozoites in the olive pythons constitutes a new host record of infection, the specific identity of the parasite remains to be determined by further studies on all endogenous developmental stages.

Several *Haemogregarina* spp. have previously been recorded in pythons from Australia but this is the first report of haemogregarines in olive pythons. The intraerythrocytic gamonts detected were similar in morphology to those of *Hg. shattocki* previously described from *Liasis amethsystinus* by Sambon and Seligmann (1907) and Mackerras (1961). However, they were larger than those of *Hg. amethsystinus* and *Hg. pythonis* described from *Liasis amethsystinus* by Johnston (1909), Johnston and Cleland (1910) and Mackerras (1961) and *Hg. fuscus* described from *Liasis fuscus* by Lewis (1913) and Mackerras (1961). Haemogregarines were also detected for the first time in a pygopodid (or snake-lizard) species, Burton's legless lizard. The gamonts detected were novel in appearance compared to other descriptions of saurian haemogregarines (cf. Mackerras 1961). In contrast, the haemogregarines detected for the first time in the robust dtella (*Gehyra robusta*) were similar in morphology to those of an un-named *Haemogregarina* sp. previously described from *Gehyra variegata* by Mackerras (1961) and Stehbens and Johnston (1967). They were much smaller than those of *Hg. heteronotae* described from *Heteronotia binoei* by Mackerras (1961) and another un-named species described from *Oedura tryoni* by Johnston (1912, 1916). The identification of individual *Haemogregarina* spp. solely on the basis of host occurrence and gamont morphology has been questioned by most comtemporary workers as little is known about their host specificities and developmental cycles. Comprehensive studies are required to rationalise the numerous species described from reptiles, amphibians and fish. These studies should endeavour to determine the sites of merogonous development in the vertebrate hosts (only gamonts occur in erythrocytes) and to identify the invertebrate vectors involved (usually leeches or arthropods).

Intraerythrocytic developmental stages of the haemosporidian parasite *Haemoproteus* were detected for the first time in a ring-tailed dragon. Several *Haemoproteus* (syn. *Haemocystidium*) spp. have been described from Australian gekkonids (Mackerras 1961; Paperna & Landau 1991) but none have previously been recorded from agamids in Australia. The trophozoites and gamonts detected in this study were similar to those of *Hp. edomensis* described from *Agama stellio* in the Middle-East by Paperna and Landau (1991) but they were smaller and less vacuolated in appearance. The only other haemosporidian parasite detected in Australian agamids was *Plasmodium giganteum* described from *Amphibolurus barbatus* by Mackerras (1961). *Haemoproteus* spp. are differentiated from *Plasmodium* spp. by the absence of merogony (schizogony) in circulating erythrocytes. *Haemoproteus* spp. undergo merogony in the vascular endothelial cells, mainly in the lungs. In this study, no schizonts were detected in erythrocytes from the ring-tailed dragon. *Plasmodium* spp. are also transmitted between vertebrate hosts by mosquito vectors whereas *Haemoproteus* spp. are transmitted by other blood-sucking vectors (midges, hippoboscid and tabanid flies). Further studies are required to determine the extent of infections in agamid lizards, to identify the parasite species involved and to determine the vector(s) involved in their transmission.

The intensity of infection by the different protozoan parasites was considered to be moderate in most animals but quite high in some, especially the ring-tailed dragon infected by *Haemoproteus* where the parasitaemia (% infected cells) approached 40%. Nonetheless, no signs of clinical disease were observed in any animals irrespective of their level of infection. All infected animals exhibited strong vital signs and their behavioural responses to capture and handling were not diminished. This suggests stable host-parasite relationships whereby hosts are able to tolerate infections and parasites do not cause significant clinical disease. Whatever the case, these particular parasites did not appear to present any major threat to the health status of the animals examined from Musselbrook Reserve.

Acknowledgments: We wish to thank The Royal Geographical Society of Queensland for organising the expedition in association with the National Parks Association of Queensland, the Queensland Department of Environment and Heritage for authorising the scientific study and The University of Queensland for providing research funds. Our special thanks to all the expedition members who assisted in the collection of samples, especially Walter Boles and Wayne Longmore from the Australian Museum for sampling birds and Louise Goggin for her courageous pursuit of fearsome serpents!

REFERENCES

Barker I.K., O'Callaghan M.G. & Beveridge I. (1989) Host-parasite associations of *Eimeria* spp. (Apicomplexa: Eimeriidae) in kangaroos and wallabies of the genus *Macropus* (Marsupialia: Macropodidae). *Int. J. Parasitol.* **19**:241-263.

Callow L.L. (1984) **Protozoal and rickettsial diseases**. Animal Health in Australia, vol. 5. Australian Bureau of Animal Health, Canberra.

Cannon L.R.G. (1967) New coccidia from Australian lizards II. *Eimeria. Parasitology* **57**:237-250.

Cleland J.B. & Johnston T.H. (1910) The haematozoa of Australian batrachians No. 1. *Proc. Roy. Soc. NSW* **44**:252-261.

Johnston T.H. (1909) On a new haemoprotozoan. *Rec. Aust. Museum* **7**:257-259.

Johnston T.H. & Cleland J.B. (1910) The haematozoa of Australian reptilia No. I. *Proc. Linn. Soc. NSW* **35**:677-685.

Johnston T.H. (1912) A census of Australian reptilian entozoa. *Proc. Roy. Soc. Qld.* **23**:233-249.

Johnston T.H. (1916) A census of the endoparasites recorded as occurring in Queensland, arranged under their hosts. *Proc. Roy. Soc. Qld.* **28**:31-79.

Lewis J.C. (1913) Report of the Veterinary and Stock Department for 1912. Blood parasites. *Rep. Admin. NT Aust.* **1912**:136-137.

Mackerras M.J. (1958) Catalogue of Australian mammals and their recorded internal parasites. *Proc. Linn. Soc. NSW* **83**:101-160.

Mackerras M.J. (1961) The haematozoa of Australian reptiles. *Aust. J. Zool.* **9**:61-122.

Paperna I. & Ostrovska K. (1989) Ultrastructural studies on sporozoite stages of *Schellackia* cf. *agamae* from liver and blood of the starred lizard *Agam stellio. Int. J. Parasitol.* **19**:13-19.

Paperna I. & Landau I. (1991) *Haemoproteus* (Haemosporidia) of lizards. *Bull. Mus. natl. Hist. nat.,* Paris **13**:309-349.

Paperna I. (1994) *Eimeria gastrosauris* n.sp. (Apicomplexa: Eimeriidae) from the stomach epithelium of Australian geckoes. *Syst. Parasitol.* **28**:15-21.

Sambon L.W. & Seligmann C.G. (1907) Descriptions of five new species of haemogregarines from snakes. *Proc. Zool. Soc. London* **1907**:283-284.

Stehbens W.E. & Johnston M.R.L. (1967) The ultrastructure of a haemogregarine parasitic in *Gehyra variegata* (Dumeril & Bibron, 1836). *Parasitology* **57**:251-261.

Appendix 1. List of native animal species examined for protozoan parasites from Musselbrook Reserve (new host records of infection are listed in bold)

		Number examined	Number infected	Parasite genus
MAMMALIA				
Chiroptera	*Taphozous georgianus* (common sheathtail bat)	2	0	
REPTILIA				
Sauria	***Amphibolurus caudicinctus* (ring-tailed dragon)**	3	**1**	***Haemoproteus***
	Amphibolurus nuchalis (central-netted dragon)	1	0	
	Lophognatus gilberti	6	0	
	Gehyra australis (northern dtella)	5	0	
	Gehyra borroloola	1	0	
	***Gehyra dubia* (dtella)**	**2**	**1**	***Eimeria***
	Gehyra nana	4	0	
	***Gehyra robusta* (robust dtella)**	7	**2**	***Haemogregarina***
	Gehyra variegata (tree dtella)	1	0	
	Heteronotia binoei (Binoe's gecko)	1	0	
	***Lialis burtonis* (Burton's legless lizard)**	1	**1**	***Haemogregarina***
	Carlia amax	1	0	
	Ctenotus lateralis	2	0	
	Ctenotus pantherinus	1	0	
	Varanus acanthurus (ridge-tailed monitor)	1	0	
Serpentes	*Achrochordus arafurae* (Arafura file snake)	1	0	
	Liasis childreni (Children's python)	4	0	
	***Liasis olivaceus* (olive python)**	3	**2**	***Haemogregarina***
			2	***Schellackia***
	Dendrelaphis punctulata (green tree snake)	1	0	
Testudines	*Elseya dentata* (northern snapping turtle)	4	0	

AMPHIBIA				
Salientia	*Bufo marinus* (cane toad)	6	0	
	Litoria caerulea (green tree frog)	6	3 *Nyctotherus*	
	Litoria inermis	1	0	
	Litoria rothii (Roth's tree frog)	1	0	
	***Litoria rubella* (desert tree frog)**	1	**1 *Nyctotherus***	
	Litoria wotjulumensis	3	0	
	Crinia remota	5	0	
AVES				
Columbiformes	*Petrophassa plumifera* (spinifex pigeon)	2	0	
Cuculiformes	*Chrysococcyx basalis* (Horsfield's bronze-cuckoo)	1	0	
	Cuculus pallidus (pallid cuckoo)	1	0	
Passeriformes	*Smicrornus brevirostris* (weebill)	1	0	
	Artamus minor (little woodswallow)	3	0	
	Cracticus nigrogularis (pied butcherbird)	1	0	
	Malurus lamberti (variegated wren)	1	0	
	Malurus melanocephalus (red-backed wren)	2	0	
	Lichenostomus flavescens (yellow-tinted honeyeater)	2	0	
	Lichenostomus keartlandi (grey-headed honeyeater)	1	0	
	Lichmera indistincta (brown honeyeater)		3	0
	Lonchura pectoralis (pictorella mannikin)	1	0	
	Poephila acuticauta (long-tailed finch)	2	0	
	Poephila guttata (zebra finch)	1	0	
Psittaciformes	*Melopsittacus undulatus* (budgerigar)	1	0	
TOTAL		98	13 (13.3%)	